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(FILE 'HOME' ENTERED AT 13:02:19 ON 20 MAR 2003)

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SEA ALPHA(W) AMYLASE(W) PROMOTER

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L1 QUE ALPHA(W) AMYLASE(W) PROMOTER

FILE 'USPATFULL, CAPLUS, BIOSIS, BIOTECHDS, MEDLINE, SCISEARCH, LIFESCI, CABA, BIOTECHNO' ENTERED AT 13:11:01 ON 20 MAR 2003

L2 93 S L1 AND (HIGH?(W)ACTI? OR IMPROV?(W)ACTI? OR HIGH?(W)EXPRESSIO
L3 48 S L2 AND AMYLOLIQUEFACIENS
L4 45 DUP REM L3 (3 DUPLICATES REMOVED)

L4 ANSWER 45 OF 45 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 1986:181105 CAPLUS
DOCUMENT NUMBER: 104:181105
TITLE: **High expression** of *Bacillus*
licheniformis .alpha.-amylase with a *Bacillus*
secretion vector
AUTHOR(S): Sibakov, Mervi
CORPORATE SOURCE: Recomb. DNA Lab., Univ. Helsinki, Helsinki, SF-00380,
Finland
SOURCE: European Journal of Biochemistry (1986), 155(3),
577-81
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A gene coding for the heat-stable .alpha.-amylase [9000-90-2] from *B. licheniformis* ATCC14580 was expressed with the aid of a *B. amyloliquefaciens* .alpha.-amylase-based expression/secretion vector by joining the structural part of the gene to a pool of vectors after the *B. amyloliquefaciens* .alpha.-amylase promoter and signal sequence. The recombinant plasmids obtained were stably maintained in *B. subtilis*, and the heat-stable .alpha.-amylase activity rose several hundred times from the level of the donor. Eight different constructions were further analyzed. Each of them had an intact *B. amyloliquefaciens* signal sequence, the only difference being in a few nucleotides beyond the C terminus of the signal peptide. This, however, was enough to cause .ltoreq.4-fold differences in protein yield. Possible reasons for the variation in the prodn. level are discussed.

Gene (15) 1987
43

L4 ANSWER 37 OF 45 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 2001-00127 BIOTECHDS

TITLE: Modified *Bacillus* **alpha-amylase promoter** having additional restriction sites near the 3' terminus for higher promotion of gene expression in *Bacillus*;
plasmid pUB-PB-MP or plasmid pUB-PBE-MP-mediated maltose-phosphorylase gene transfer and expression in *Bacillus subtilis* for foodstuff and drug production

AUTHOR: Inoue Y; Fushimi N; Mizubuchi H; Yamamoto Y; Ohshima Y; Yasutake N; Miyoshi S

PATENT ASSIGNEE: Showa-Sangyo

LOCATION: Tokyo, Japan.

PATENT INFO: WO 2000053778 14 Sep 2000

APPLICATION INFO: WO 2000-JP1415 8 Mar 2000

PRIORITY INFO: JP 1999-286034 6 Oct 1999; JP 1999-60904 8 Mar 1999

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2000-594327 [56]

AB A promoter sequence having **higher activity** than the alpha-amylase gene promoter of a *Bacillus* sp. strain obtained by inserting near the 3' terminus of the promoter 1 or more additional restriction enzyme cleavage sites, is claimed. Also claimed are: a cassette expressing the novel promoter; a gene expression vector containing the cassette as above; microbial cells transformed by the above vector; and preparation of the gene expression product by culture of the cells as above. The above is used for expression of microbial genes at higher promotion efficiency, for the production of proteins for use as drugs or foodstuffs at lower cost. In an example, polymerase chain reaction with specified DNA primers were used to amplify the *Bacillus amyloliquefaciens* (IFO-15535) **alpha-amylase promoter** to obtain a modified promoter fragment (0.25 kb) having a BamHI restriction cleavage site near the 3' end. Using a Different DNA primer set, a fragment (0.27 kb) with BamHI, SmaI, KpnI, SacI and EcoRI restriction sites was produced. These fragments were inserted into plasmid pUB110 which was used to transform *Bacillus subtilis* 168 to produce recombinant maltose-phosphorylase. (48pp)